9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

9.1 Availability of Other *In Vitro* AR TA Data

Some of the peer-reviewed publications identified during the initial literature search for AR TA studies were not abstracted for inclusion in this BRD. The reasons for not abstracting these publications include:

- The studies lacked appropriate qualitative and/or quantitative test data;
- The test substances were not adequately identified, or were undefined mixtures; or,
- The publications contained insufficient information about the test method used.

NICEATM made a formal request in the *Federal Register* (Vol. 66, No. 57, pp. 16278 – 16279) for unpublished AR TA data and/or information from completed studies using or evaluating AR TA assays. A submission was received from Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan, in response to this request. The data from this submission are included in **Appendix D,** which also contains the *in vitro* AR TA data from the 26 peer-reviewed publications considered in this BRD.

Some companies involved in pharmaceutical discovery and development routinely use *in vitro* AR TA assays to screen substances for their potential androgenic activity. However, these data are not in the public domain and have not been provided to NICEATM.

While every effort was made to include all available, pertinent *in vitro* AR TA data in this BRD, the authors recognize that some data may have been inadvertently excluded.

9.2 Conclusions from Other Scientific Reviews of *In Vitro* AR TA Methods

To date, no independent peer reviews of *in vitro* AR TA assays have been conducted. However, two recent workshops addressed the use of these assays as potential endocrine disruptor screening methods. Although the strengths and limitations of AR TA assays were discussed at both workshops, no effort was made to evaluate the reliability and performance of the assays. The conclusions from these workshops are summarized below.

9.2.1 1996 Endocrine Disruptor Screening Methods Workshop

In vitro AR TA assays were discussed at an Endocrine Disruptor Screening Methods Workshop held in July 1996 at Duke University in Durham, North Carolina. Gray et al. (1997) edited the proceedings of this workshop, which was cosponsored by the U.S. EPA, the Chemical Manufacturers Association (CMA), and the World Wildlife Fund (WWF).

An assessment was made of *in vitro* AR TA assays that use monkey kidney CV-1 cells. For these assays, CV-1 cells are transiently transfected with an expression vector containing cDNA for human AR and a reporter vector containing a reporter gene, typically luciferase, linked to an AR-inducible response element. The major advantages of these assays, as described by the authors, include:

- The use of human AR;
- CV-1 cells have some metabolic activity; and
- The assays can distinguish between agonists and antagonists.

The major disadvantages cited by the authors include:

- Transient co-transfections of expression and reporter vectors can be difficult to prepare and maintain;
- The assay requires both AR expression and reporter vectors;
- Reproducibility of the assay requires strict adherence to the protocol; and
- Metabolism of a test substance during the required 48-hour incubation period may confound results.

In addition, Gray et al. (1997) discussed the major advantages and disadvantages of yeast-based AR TA assays. These assays use recombinant, stably transformed yeast cells that contain AR from humans or other species of interest, and a reporter gene, typically β-galactosidase, linked to an AR-inducible response element.

The major advantages of yeast assays, as described by the authors, include:

- They are relatively easy to perform;
- A short incubation time, ranging from 4 hours to overnight, is used;

- Large number of samples can be processed relatively quickly; and
- Substances can be tested over a wide dose range.

The major disadvantages of yeast-based AR TA assays cited by the authors include:

- They do not appear to distinguish between agonists and antagonists (e.g., the known AR antagonist, hydroxyflutamide, is reported to induce TA in yeast-based AR assays);
- There may be significant metabolic differences between yeast and mammalian cells that could make it difficult to extrapolate data from these assays to humans;
- The cell wall and chemical transport systems of yeast cells are reported to selectively maintain low intracellular concentrations of some steroid hormones, a phenomenon that may apply to other types of substances;
- The porosity of the yeast cell wall versus that of mammalian cell membranes may be significantly different;
- The assay gives negative and/or weak positive results for *p,p'*-DDE, a substance that binds strongly to rat AR and hAR in COS and CV-1 cells.

9.2.2 1997 Workshop on Screening Methods for Detecting Potential (Anti-) Estrogenic/Androgenic Chemicals in Wildlife

In March 1997 the U.S. EPA, the CMA, and the WWF cosponsored a workshop in Kansas City, Missouri, that addressed the use of "gene expression" assays as a type of *in vitro* screening methods for detecting potential (anti-)androgenic substances in wildlife. Ankley et al. (1998) edited the proceedings of this workshop.

The major advantages described by the authors for using gene expression assays as endocrine disruptor screens for wildlife include:

- Assays that use eukaryotic cell lines can distinguish between agonists and antagonists;
- The assays are amenable to automation using microtiter plates, which would allow for the rapid processing of large numbers of samples; and
- The methods are amenable to standardization.

The major disadvantages include:

- Require specialized equipment and training;
- Transient transfection of plasmids can be labor-intensive and may contribute to increased interassay variability;
- Poor correlation of results for some substances tested in yeast-based assays versus those using mammalian cells;
- These assays currently have limited applicability to nonmammalian species, which have been poorly studied with regard to development of suitable reporter gene assays for detection of (anti-)androgenic substances.